Electrochemical Detection of 17β-estradiol by using DNA Aptamer Immobilized Nanowell Gold Electrodes

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Abstract

Aptamer is the single-stranded oligonucleotide which binds to various target molecules such as proteins, peptides, lipids and small organic molecules with high affinity and specificity. DNA aptamers specific for the 17β-estradiol were selected by SELEX (Systematic Evolution of Ligands by EXponential enrichment) process from a random DNA library. These DNA aptamers have a high affinity to 17β-estradiol as an endocrine disrupting chemical. Nanowell and 200μm gold electrode were used as substrate for DNA aptamer immobilization and electrochemical analysis. Especially, nanowell gold electrode was fabricated by e-beam lithography. The size of single nanowell is 130nm and 40,000 nanowells were deposited on one gold electrode. The immobilization method was based on the interaction between the biotinylated aptamer and streptavidin deposited on gold electrode previously. Immobilization procedure was optimized by surface plasma resonance (SPR) and electrochemical analysis.

After the immobilization of DNA aptamer on streptavidin modified gold electrode, 17β -estradiol solution was treated on aptamer immobilized gold electrode. The current of gold electrode was decreased by the binding of 17β-estradiol to DNA aptamer immobilized on gold electrode. However, in negative control experiments of 1-aminoanthraquinone and 2-methoxynaphthalene, the current was rarely decreased. And more sensitive data was obtained from nanowell gold electrode comparing with 200 μm gold electrode.

Introduction

In resent years, studies for oligonucleotides (aptamers) binding to specific target compounds were reported. Their ability of biomolecular recognition for target molecule has opened new trends in affinity biosensing because the signal of biosensor depends on the specificity and the affinity of the molecular recognition element [1]. In vitro selected aptamers are reproducibly synthesized easily and economically. In addition, aptamers are not restricted for various targets such as proteins, peptides, amino acids, nucleotides, drugs, carbohydrates and other small organic and inorganic compounds. Therefore, aptamers are good alternatives as sensing molecules for the development of chemical sensing system for environmental toxicants. Biosensors for detecting target compounds are composed of a biological recognition element and a physical transducer that produces a useful signal from interaction between aptamers and target compounds. Optical, electrochemical or mass-sensitive devices to generate light, current or frequency signal are commonly used systems in aptasensors. However, in biosensor system for small organic chemicals detection, mass-sensitive detection methods such as Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM) and cantilever device are not proper because the molecular weight of target chemicals like toxicants or endotoxins is too small to produce a signal [2]. Also optical methods with fluorescence or radioactive tags have some disadvantages in terms of cost and experimental complexity. They need the labeling of dyes or tags and expensive optical detection devices [3]. Electrochemical methods are well suited for small organic chemical sensing. Electrochemical detection system is label-free system, electrochemical signal is directly produced from biorecognition and there is no need for expensive signal transduction equipment. Additionally, current change is not related to the mass of target compound [4]. The ssDNA aptamer, binding to 17β -estradiol, endocrine disrupting chemicals (EDCs), was isolated by SELEX process. It was immobilized on gold electrode based upon avidin-biotin interaction and electrochemical analysis with aptamer immobilized gold electrode chip was carried out for 17β-estradiol detection. The immobilization procedure of ssDNA aptamer on streptavidin modified gold electrode was optimized by SPR system. Cyclic Voltametry (CV) and Square Wave Voltametry (SWV) were measured for electrochemical detection.

Materials and Methods

ssDNA aptamer immobilization on gold electrode

To construct aptamer based biosensor depending on electrochemical detection system, biotinylated ssDNA aptamer was immobilized on streptavidin modified gold electrode. At first, Self Assembly Monolayer (SAM) was taken through incubation of HS---COOH (3,3'-dithioldipropionic acid) for 30min. Treated EDC(1mM) / NHS(1mM) made activate COOH group for peptide bonding with amino terminal of streptavidin. Streptavidin (Sigma Chemicals Co.) solution (10ug/ml) was incubated for 1hr. Ethanolamine (1mM, pH 8.5) was used to block the non-reacted activated COOH group. Finally, biotinylated ssDNA aptamer was binding to streptavidin depending on avidin-biotin interaction. This procedure was optimized by Surface Plasmon Resonance (SPR) experiment and electrochemical analysis system.

Electrochemical Measurement

Model 1030 electrochemical analyzer (ALS/CH Instrument, USA) was used for electrochemical detection. Sensing block is composed of block body, reference electrode (Ag/AgCl), counter electrode (Pt) and working electrode (gold electrode chip). Ferrocyanide solution was used as a mediator to produce electron flow. One chip has eight electrodes and ssDNA aptamer is immobilized on each modified gold electrodes. After sample was treated on aptamer immobilized gold electrode for 30min, cyclic voltametry (CV) and square wave voltametry (SWV) were performed in 5mM $K_3Fe(CN)_6$ solution containing 0.1M KCl. CV was measured in the potential range -0.3 to 0.6 V with scan rate of 200 mV/s. SWV was performed with a pulse amplitude setting of 25 mV and pulse width setting of 5ms. Electrons are flowed by the redox between ferrocyanide and ferricyanide.

Results and Discussion

In order to confirm the procedure for the immobilization of biotinylated ssDNA aptamer on streptavidin modified gold electrode, an electrochemical analysis was carried

out. CV and SWV values were measured range -0.3 ~ 0.6 V in 5mM $K_3Fe(CN)_6$ solution containing 0.1M KCl before and after biotinylated ssDNA aptamer was immobilized on streptavidin modified gold electrode. The SWV signal can provide a larger electrochemical change than the CV signal because the SWV signal can suppress the capacitive current later, which is due to charging effects in the protein layer. In the SWV data for aptamer immobilization, red line is signal before aptamer immobilization and blue line means signal after aptamer immobilization. The current change was proportional to treated ssDNA aptamer concentration. It is reasonable because biotinylated ssDNA interfere the electrons flow produced from the redox between ferrocyanid and ferricyanide.

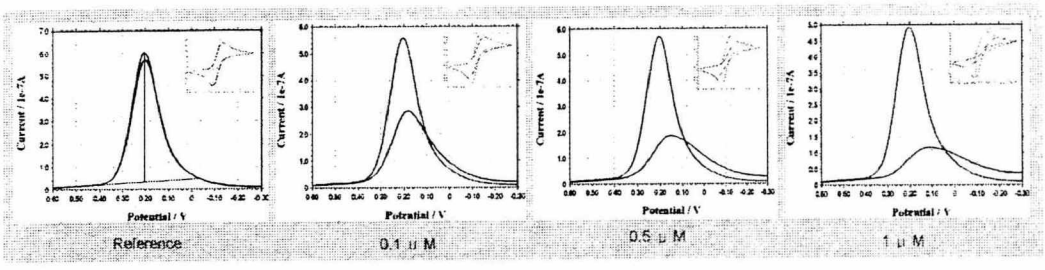

Figure 1. Red line is a current before DNA aptamer immobilization, blue line is a current after DNA aptamer immobilization. ssDNA aptamer immobilization 200μm gold electrode. Current decreasing was proportional to immobilized DNA aptamer concentration.

Three kinds of small organic chemical were tested for electrochemical detection of 17β -estradiol. 2-methoxynaphthalene and 1-aminoanthraquinone were tested as a negative control to estimate the specificity of this biosensor system for 17β-estradiol. The CV and SWV analysis was performed before and after 17β-estradiol treatment on ssDNA aptamer immobilized gold electrode. The electrode potential after 17β-estradiol treatment was shifted based on the oxidation peak position before 17β-estradiol treatment. Also the redox reaction was demonstrated by the peaks in both the oxidation and the reduction curves. The current was decreased after 17β-estradiol treatment. But there were rarely current change and potential shift for 1-aminoanthraquinone, negative control. We can suggest from these data that only 17β-estradiol bound to ssDNA aptamer immobilized gold electrode specifically.

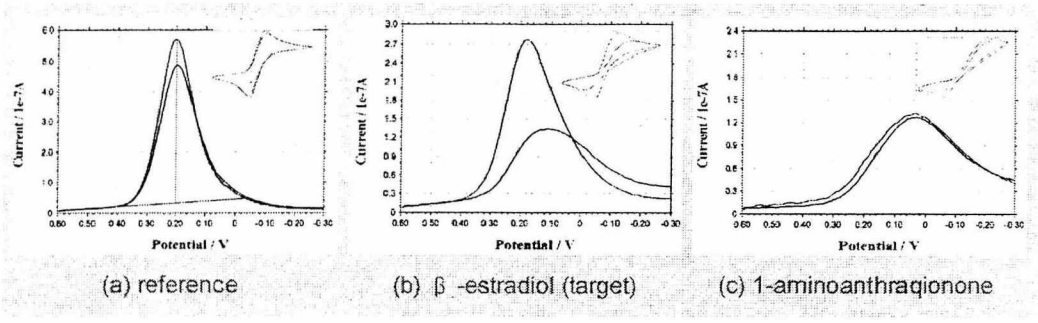

Figure 2. Red line is signal before sample treated and blue line is signal after sample treated. (a) is the SWV data of reference. In reference channel aptamer and target were not treated. (b) is the SWV result of β-estradiol binding. The current was decreased due to β-estradiol binding. (c) Is the result of 1-aminoanthraqionone, negative control. The current was rarely deceased by this.

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References

1. E. Luzi, M. Minunni, S.Tombelli, M. Mascini (2003) New trends in affinity sensing: aptamers for ligand binding, *Trends in Analytical Chemistry* 22, 810.
2. J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.-J. Guntherodt, Ch. Gerber, J. K. Gimzewski (2000) Translating Biomolecular Recognition into Nanomehcanics, *Science* 288, 316.
3. Epstein, J.R., Biran, I. & D.R.(2002) Fluorescence-based nucleic acid detection and microarrys, *Analytical Chimica Acta* 469, 3.
4. T Gregory Drummond, Micheal G Hill, Jacqueline K Barton (2003) Electrochemical DNA sensor, *Nature Biotechnology* 21, 1192.